A PHARMACEUTICAL COMPOSITION FOR TREATING RHEUMATISM AND THE PREPARATION THEREOF

CROSS-REFERENCE TO RELATED APPLICATION

This application is a National Phase Patent Application and claims the priority of International Application Number PCT/CN2002/00246, filed on April 9, 2002.

THE FIELD OF THE INVENTION

The invention is directed to a medicine for treating rheumatism, and the medicine's preparation.

THE BACKGROUND OF THE INVENTION

It is believed that rheumatoid arthritis (RA) is refractory and about 18,000,000 RA patients have been disabled because of this disease. The medical research into curing RA has continued for about a century. Aspirin is the first medicine which was widely used to treat RA. The medicine to treat RA can be divided into 2 kinds: non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressive agents. NSAIDs includes cyclophthasine, antinfan and adrenal cortex hormone. Clinical research has proven the effectiveness of NSAIDs. Immunosuppressive agents include methotrexate, cyclophosphane, penicillamine among others. Immunoregulation has become one of the important therapies in recent years. But all the medicines used to treat rheumatism have serious side-effects. A medicine that can treat rheumatism effectively and nontoxically has not been invented before the present invention.

There are 3 directions in the research of antirheumatics that should

be emphasized. The first direction is NSAIDs and cytokine-antagonists, such as recombinant, soluble TNFα antagonists, IL-1 inhibitors and PAF (platelet-activating factor) inhibitors. The second direction is the new immunosuppressive agents and immunomodulators, such as cyclosporin A. The third direction is the compound medicines.

In traditional Chinese medicine (TCM), the research on "Bi Zheng" (equivalent to the definition of rheumatism or arthralgia in the modern medicine) can be traced back to the Han dynasty more than 1,500 years ago. Three prescriptions: "Ma Xing Shi Gan decoction", "Fangji Huangqi decoction" and "Wutou decoction" were used to treat "Bi Zheng" as recorded in the medical classics "Shanghan Lun" written by the famous doctor Zhang Zhongjing at that time. A wild plant called "huo ba hua" (Gelsemium elegans Bentll) in Sichuan province has been proven effective in the treatment of rheumatism based on clinical research performed in the local area (Sichuan province). However, subsequent study found that it had a serious side-effect on the reproductive organs and other uncontrollable problems.

The treatment of "Bi Zheng" by the methods of TCM has reached a high level after a long history of development by numerous doctors. Currently, there are many effective prescriptions and herbs. More than 80 kinds of herbs and 29 kinds of patent medicines are recorded in the China pharmacopoeia 1995 edition and 2000 edition. However, many problems still remain: for example, ① TCM is still ineffectual in treating serious arthralgia such as rheumatoid arthritis; ② the dosage forms cannot meet the needs of modern life. ③ some medicine have good effects, but the side-effects are too damaging, such as when using the

extract of *triperygium wilfordii*. Thus, it is necessary to develop a new antirheumatic medicine that is highly-effective, with minimal noxiousness, and convenient to administer. This medicine should have similar effects and lower side-effects than artificial, anti-rheumatic medicine.

SUMMARY OF THE INVENTION

The invention provides an antirheumatic that is highly-effective, has minimal noxiousness, and is convenient to administer, and its preparation thereof.

The medicine uses the following crude herbs:

Tripterygium hypoglaucum (Levl.) Hutch;

Epimedium brevicornum Maxim;

Lycium barbarum L; and,

Cuscuta chinensis Lam (or *Cuscuta australis* R. Br.)

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

The antirheumatic medicine of the present invention utilizes the crude herbs as follows:

Tripterygium hypoglaucum (Levl.) Hutch;

Epimedium brevicornum Maxim;

Lycium barbarum L; and,

Cuscuta chinensis Lam (or *Cuscuta australis* R. Br.)

The crude herbs to produce the antirheumatic medicine can be combined in several ways. The *tripterygium hypoglaucum* (Levl.) Hutch is the necessary herb with one or two or three of the other three herbs added tomake the medicine.

One of the optimal proportional combinations of crude herbs for the medicine is as follows:

Tripterygium hypoglaucum (Levl.) Hutch.	1-4 parts by weight
Epimedium brevicornum Maxim.	1-4 parts by weight
Lycium barbarum L.	1-4 parts by weight
Cuscuta chinensis Lam.	1-4 parts by weight

A second optimal proportional combination of crude herbs for the medicine is as follows:

Tripterygium hypoglaucum (Levl.) Hutch.	2 parts by weight
Epimedium brevicornum Maxim.	2 parts by weight
Lycium barbarum L.	1 parts by weight
Cuscuta chinensis Lam.	1 parts by weight

The third optimal proportional combination of crude herbs for the medicine is as follows:

Tripterygium hypoglaucum (Levl.) Hutch.	1-4 parts by weight
Epimedium brevicornum Maxim.	1-4 parts by weight

The fourth optimal proportional combination of crude herbs for the medicine is as follows:

Tripterygium hypoglaucum (Levl.) Hutch.	2 parts by weight
Epimedium brevicornum Maxim.	2 parts by weight

The fifth optimal proportional combination of crude herbs for the medicine is as follows:

Tripterygium hypoglaucum (Levl.) Hutch	1-4 parts by weight
Epimedium brevicornum Maxim	1-4 parts by weight
Lycium barbarum L	1-4 parts by weight

The sixth optimal proportional combination of crude herbs for the medicine is as follows:

Tripterygium hypoglaucum (Levl.) Hutch 2 parts by weight
Epimedium brevicornum Maxim 2 parts by weight
Lycium barbarum L 1 parts by weight

The seventh optimal proportional combination of crude herbs for the medicine is as follows:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 parts by weight
Epimedium brevicornum Maxim 1-4 parts by weight
Cuscuta chinensis Lam 1-4 parts by weight

The eighth optimal proportional combination of crude herbs for the medicine is as follows:

Tripterygium hypoglaucum (Levl.) Hutch 2 parts by weight
Epimedium brevicornum Maxim 2 parts by weight
Cuscuta chinensis Lam 1 parts by weight

The content of the icariin ($C_{33}H_{40}O_{15}$) in the medicine above should not be less than 2.0 mg.

The optimal proportional combinations of crude herbs for the medicine can be derived in other ways as follows:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 parts by weight
Lycium barbarum L 1-4 parts by weight
And / or *Cuscuta chinensis* Lam 1-4 parts by weight

The optimal proportional combinations of crude herbs for the medicine can be derived in another way as follows:

Tripterygium hypoglaucum (Levl.) Hutch 2 parts by weight
Lycium barbarum L 1 part by weight
And / or *Cuscuta chinensis* Lam 1 part by weight

The crude herbs are prepared based on their proportional combinations and then they can be made into any dosage forms used in

the clinic, such as the bolus form, the powder forms, the ointment forms, the tablet forms, the soft or hard capsule forms, the granule forms, the injection forms and so on.

The preparation method of the invented medicine is as follows:

The crude herbs are prepared based on the proportional weight:

Tripterygium hypoglaucum (Levl.) Hutch	1-4 parts by weight
Epimedium brevicornum Maxim	1-4 parts by weight
Lycium barbarum L	1-4 parts by weight
Cuscuta chinensis Lam	1-4 parts by weight

The *Tripterygium hypoglaucum* (Levl.) Hutch. and *Epimedium brevicornum* Maxim are broken into pieces. Then the pieces are decocted by water for 2～4 times separately. The *Lycium barbarum* L and *Cuscuta chinensis* Lam are soaked in the hot water (80~95℃) for 1～3 times separately. The decoction fluid and the immersion fluid of the herbs are collected and added separately to the corresponding column of adsorbent resins having macroscopic voids. After adsorption, the columns are washed with water until the flushing liquid turns clear. Then the resins are eluted with 60%-80% alcohol. The eluted liquids are collected from the time when their color turns deep until their color turns very weak. Then the remaining alcohol in the column is pushed out by high pressure water and is added to the eluted liquids. The combined eluted liquids are about 3～8 times more concentrated than the corresponding crude herb in terms of the effective compounds. All 4 eluted liquids are condensed to a specific density of 1.10. The condensed liquors are dried by a spray drying method to get the extract of the crude herbs. The 4 kinds of extracts are mixed uniformly to be made into

approproate dosage forms that are needed by the clinic.

The optimal preparation method of the invented medicine is as follows:

The crude herbs are prepared based on the proportional weight:

Tripterygium hypoglaucum (Levl.) Hutch	2 parts by weight
Epimedium brevicornum Maxim	2 parts by weight
Lycium barbarum L	1 part by weight
Cuscuta chinensis Lam	1 part by weight

The *Tripterygium hypoglaucum* (Levl.) Hutch. is broken into pieces. Then the pieces are added with 13, 10, 10 volume weight of water to decoct 3 times respectively. Each time is for 1 hour. The *Epimedium brevicornum* Maxim is cut into pieces. Then the pieces are added with 15, 10, 10 volume weight of the water to decoct 3 times respectively. Each time is for 1 hour. The *Lycium barbarum* L is broken into coarse powder and soaked in hot water (80℃, 20 volume weight of the crude herb) 3 times. Each time is for 1 hour. The *Cuscuta chinensis* Lam is broken to coarse powder and soaked in the hot water (80℃, 31 volume weight of the crude herb) 3 times. Each time is for 1 hour. The decoction fluid and the immersion fluid of the herbs are filtrated separately and added to the corresponding column of adsorbent resins having macroscopic voids (the type of the resin is JD-1 (WLD resin)). After adsorption, the resins in the columns are eluted with 70% alcohol. The eluted liquids are collected from when the color of the liquid turns deep until the color of the liquid turns very weak. The alcohol is separated from the eluted liquid. Then the remaining eluted liquid is condensed and dried to obtain the extract powder. The 4 kinds of extract

powders are mixed uniformly to be made into any dosage forms that are needed by the clinic.

The invented medicine can be prepared by the method as follows:

The crude herbs are prepared based on the proportional weight described above. The *Tripterygium hypoglaucum* (Levl.) Hutch. and *Epimedium brevicornum* Maxim are cut into pieces. The *Lycium barbarum* L and *Cuscuta chinensis* Lam are crushed or not crushed. The 4 kinds of herbs are extracted using- 0~95% alcohol **at 10 ~ 98℃** for 1~4 times separately or together. The extracted liquids are mixed or not mixed. Then the extracted liquids are condensed, dried, broken into pieces and mixed uniformly. The mixed powder can be made into any dosage form needed in the clinic.

The invented medicine can be made from the effective constituents of the 4 herbs.

The effective constituents of *Epimedium brevicornum* Maxim are icariin, icariside I , icariside II, and Icariin A. The effective constituents of *Tripterygium hypoglaucum* (Levl.) Hutch. are diterpenes, triterpenes and alkaloids compounds. The effective constituents of *Lycium barbarum* L and *Cuscuta chinensis* Lam are both flavones.

The crude herb *Epimedium brevicornum* Maxim can be replaced by one or more kinds of the effective constituents of itself, such as icariin, icariside I , icariside II, and Icariin A. The crude herb *Tripterygium hypoglaucum* (Levl.) Hutch. can be replaced by one or more kinds of the effective constituents of itself, such as diterpenes, triterpenes and alkaloids compounds. While the *Lycium barbarum* L and *Cuscuta chinensis* Lam can be replaced by flavones.

It has been proven by pharmacodynamics research that the invented medicine (Fengshiping Capsule) can inhibit the primary and secondary injury adjuvant arthritis (AA). It can inhibit the delayed hypersensitivity (DTH) in the ear of a mouse caused by the 2,4 dinitrofluorobenzene (DNFB). It can inhibit the production of hemolysin antibody and the activity of the IL-1, IL-2, IL-6 and TNF in the macrophage and splenocyte. The Fengshiping Capsule can inhibit the lymphocyte transformation induced by the ConA. It can inhibit the CD_4、CD_8 cells remarkably, especially CD_4 cells, but it does not affect the relative proportion of CD_4/CD_8 very much. There was a remarkable linear relationship between the dosage and the effect. Twelve to eighteen (12~18) g (crude medicine)/kg was the minimum effective dose. The invented medicine can inhibit the activity of the NK (Natural Killer) cells. At the effective dose, Fengshiping Capsule did not cause the atrophy of the important immune organs such as thymus and spleen, and did not inhibit the phagocytic activity of the macrophage.

The invented medicine had a remarkable anti-inflammatory action. It can inhibit the excessive penetrating damage of the capillary in the mouse's abdominal cavity caused by the injection of acetic acid. It can inhibit the swelling in the ear of the mouse caused by the croton oil. It can inhibit the pleuritis in the mouse and the assembling of the WBC (white blood cells) to the CMC cyst in the rat induced by the carrageenan. But the invented medicine cannot obviously inhibit the rat's foot swelling induced by the carrageenan and the granuloma caused by the tampon. The Fengshipng Capsule can remarkably inhibit the body-twist reaction caused by the acetic acid in the mouse.

Experimental example 1: the effect on the adjuvant arthritis (AA)

1.1 The preventive effect on the AA of the invented medicine

Seventy-two (72) isogenous SD rats of the same batch, half male and half female, 180 ~ 220g weight each, were divided randomly into 6 groups. Each group had 12 rats. Six (6) rats lived in a cage. The perimeter of the double ankle joints and the feet of the rats were measured accurately and recorded as the normal value. All the rats were given orally the same volume of the invented medicine at different concentrations of the solution with tragacanth. One (1) hour later, all the rats were injected with 0.1ml Freund's complete adjuvant (FCA) under the skin of the left posterior limb. In the next 30 days, all the rats were given orally the correspondent medicine once a day at the same dosage. And on these days, the perimeters of the double ankle joints and the feet of the rats were measured once a day. In this experiment, the swelling degree (△cm) equals the difference value of the perimeters measured after the FCA injection and before the FCA injection. (See the result in table 1.1 and 1.2) At the end of the experiment, the major organs of the rats were weighed. (See the table 1.3, 1.4)

Table 1.1 The effect of the Fengshiping on the swelling degree of the left ankle joint and foot after the injection of FCA in the rat AA model ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)						
		1d	2d	3d	9d	12d	14d	16d
Control	-	0.69±0.17	0.69±0.12	0.92±0.18	0.84±0.41	1.10±0.30	1.65±0.68	2.10±0.55
Fengshiping	7.5	0.74±0.12	0.66±0.074	0.83±0.13	0.77±0.27	1.11±0.45	1.34±0.53	1.91±0.61
Fengshiping	15	0.80±0.24	0.62±0.13	0.76±0.18	0.49±0.17*	0.73±0.34*	1.00±0.48*	1.38±0.67*
Fengshiping	30	0.75±0.19	0.67±0.19	0.87±0.28	0.63±0.22	0.73±0.34*	0.82±0.43**	1.05±0.53**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.72±0.11	0.68±0.16	0.91±0.18	0.66±0.23	0.88±0.29	1.03±0.36*	1.37±0.33*
prednisone	0.01	0.64±0.14	0.64±0.16	0.50±0.26	0.46±0.25	0.72±0.46*	0.87±0.46**	1.28±0.69*

Group	Dose (g/kg)	Swelling degree(△cm)					
		18d	20d	22d	24d	26d	28d
Control	-	2.18±0.44	2.05±0.46	2.00±0.46	2.04±0.57	1.92±0.65	1.83±0.67
Fengshiping	7.5	1.74±0.73	1.81±0.55	1.81±0.52	1.77±0.55	1.65±0.55	1.55±0.49
Fengshiping	15	1.32±0.59**	1.28±0.58**	1.34±0.61*	1.33±0.67*	1.20±0.64*	1.08±0.58**
Fengshiping	30	0.95±0.50**	0.87±0.51**	0.95±0.54**	0.89±0.59**	0.90±0.57**	0.86±0.51**
Tripterygium hypoglaucum (Levl.) Hutch.	5	1.47±0.43**	1.50±0.43**	1.49±0.43*	1.42±0.53*	1.40±0.56*	1.32±0.57
prednisone	0.01	1.18±0.7**6	1.03±0.67**	1.05±0.69*	0.90±0.64**	0.86±0.65**	0.85±0.59**

Comparing to the control group *P<0.05 , **P<0.01(the signs have the same meaning in the following tables)

1.2 The effect of the Fengshiping on the swelling degree of the left ankle joint and foot after the injection of FCA in the rat AA model ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)					
		2d	9d	12d	14d	16d	18d
Control	-	0.14±0.05	0.06±0.10	0.34±0.36	0.80±0.52	1.43±0.67	1.36±0.61
Fengshiping	7.5	0.18±0.06	0.10±0.14	0.26±0.36	0.82±0.52	1.31±0.64	1.28±0.71
Fengshiping	15	0.15±0.08	0.02±0.06	0.13±0.10*	0.37±0.31*	0.90±0.56*	0.79±0.60*
Fengshiping	30	0.18±0.09	0.06±0.06	0.16±0.08*	0.29±0.20**	0.49±0.41**	0.33±0.29**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.16±0.07	0.01±0.07	0.11±0.10	0.44±0.19**	0.87±0.56*	0.84±0.67*
prednisone	0.01	0.20±0.06	0.08±0.08	0.21±0.16	0.44±0.43	0.99±0.63	0.84±0.74*

Group	Dose (g/kg)	Swelling degree (△cm)				
		20d	22d	24d	26d	28d
Control	-	1.28±0.57	1.38±0.64	1.35±0.75	1.20±0.78	1.12±0.63
Fengshiping	7.5	1.33±0.71	1.31±0.73	1.27±0.73	1.16±0.73	1.07±0.65
Fengshiping	15	1.74±0.57*	1.92±0.61*	0.95±0.64*	0.88±0.58*	1.83±0.55
Fengshiping	30	0.27±0.30**	0.34±0.31**	0.32±0.33**	0.31±0.32**	0.34±0.32**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.82±0.65*	0.89±0.70*	0.80±0.67*	0.83±0.68	0.75±0.69
prednisone	0.01	0.82±0.72*	0.79±0.74*	0.75±0.67**	0.68±0.64*	0.71±0.67

1.3 The effect of the Fengshipng on the body weight of the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Body weight change(g)		
		Initial BW	BW at 1 month later	BW change
Control	-	228±34	231±52	3
Fengshiping	7.5	229±34	220±46	-9
Fengshiping	15	223±40	232±34	9
Fengshiping	30	224±37	256±60	32
Tripterygium hypoglaucum (Levl.) Hutch.	5	226±45	230±43	4
prednisone	0.01	264±55	244±31	-21

1.4 The effect of the Fengshiping on the organ weight of the immune system in the AA rats (prevention experiment)($\overline{X} \pm S$)

Group	Dose (g/kg)	Organ index [(organ weight/body weight)/100]			
		Liver	Spleen	Thymus	adrenal gland
Control	-	3.92±0.65	0.34±0.10	0.098±0.040	0.027±0.01
Fengshiping	7.5	3.73±0.29	0.31±0.09	0.078±0.038	0.027±0.008
Fengshiping	15	3.48±0.32	0.38±0.10	0.100±0.034	0.023±0.005
Fengshiping	30	3.38±0.28*	0.44±0.12*	0.100±0.032	0.022±0.007
Tripterygium hypoglaucum (Levl.) Hutch.	5	3.21±0.30**	0.36±0.05	0.052±0.011**	0.026±0.009
prednisone	0.01	3.04±0.20**	0.32±0.08	0.050±0.060**	0.020±0.004*

1.2 The therapeutic effect on the AA of the invented medicine

Fifty (50) male SD rats were divided into 5 groups at random. The experimental model was the same as the prevention experiment, but the correspondent medicines were given orally 13 days after the injection of the FCA. The medicines were given once a day for 2 weeks. The degree of swelling (△cm) was the difference of the perimeters between the value of first administration day and the other days. (See the result in table 1.5, 1.6) The major organs' weight is showed in table 1.7.

1.5 The therapeutic effect of Fengshiping on the degree of swelling of the left anklejoint and foot in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)			
		1d	2d	4d	6d
Control	-	1.81±0.27	1.92±0.19	2.12±0.22	2.16±0.27
Fengshiping	7.5	1.68±0.50	1.64±0.54	1.70±0.57	1.82±0.61
Fengshiping	15	1.44±0.41*	1.51±0.36**	1.65±0.34**	1.74±0.31**
Fengshiping	30	1.50±0.56	1.48±0.41**	1.51±0.44**	1.59±0.51**
prednisone	0.01	1.78±0.51	1.70±0.51	1.63±0.50*	1.58±0.50**

Group	Dose (g/kg)	Swelling degree (△cm)			
		8d	10d	12d	14d
Control	-	1.92±0.32	1.87±0.34	1.92±0.39	1.78±0.44
Fengshiping	7.5	1.67±0.68	1.60±0.71	1.61±0.77	1.58±0.71
Fengshiping	15	1.46±0.37**	1.48±0.30*	1.28±0.37**	1.22±0.38**
Fengshiping	30	1.29±0.58**	1.29±0.65**	1.26±0.67**	1.20±0.68*
prednisone	0.01	1.27±0.46**	1.09±0.54**	0.94±0.50**	0.94±0.42**

1.6 The therapeutic effect of Fengshiping on the degree of swelling of the right anklejoint and foot in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)			
		2d	4d	6d	8d
Control	-	0.36±0.26	0.45±0.25	0.55±0.34	0.47±0.29
Fengshiping	7.5	0.12±0.25	0.34±0.32	0.48±0.41	0.28±0.38
Fengshiping	15	0.21±0.18	0.38±0.27	0.44±0.33	0.21±0.33*
Fengshiping	30	0.10±0.48	0.06±0.28**	0.11±0.24**	0.06±0.27**
prednisone	0.01	0.10±0.13*	0.15±0.28*	0.11±0.25**	-0.08±0.34**

Group	Dose (g/kg)	Swelling degree(△cm)		
		10d	12d	14d
Control	-	0.48±0.25	0.46±0.31	0.40±0.36
Fengshiping	7.5	0.35±0.30	0.30±0.29	0.30±0.35
Fengshiping	15	0.19±0.45*	0.06±0.31**	-0.06±0.34**
Fengshiping	30	0.02±0.39**	0.05±0.38*	-0.02±0.41**
prednisone	0.01	-0.13±0.28**	-0.26±0.36**	-0.33±0.39**

n = 10 , comparing with the control group , *P<0.05 , **P<0.01

1.7 The effect of the Fengshiping on the organ weight of the immune system in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Organ index [(organ weight/body weight)/100]			
		Liver	Spleen	Thymus	adrenal gland
Control	-	0.35±0.23	0.35±0.061	0.073±0.014	0.026±0.0071
Fengshiping	7.5	3.21±0.52	0.33±0.091	0.071±0.026	0.024±0.0085
Fengshiping	15	3.40±0.54	0.36±0.014	0.067±0.022	0.023±0.0048
Fengshiping	30	2.79±0.43	0.32±0.083	0.069±0.029	0.023±0.0072
Tripterygium hypoglaucum (Levl.) Hutch.	5	3.92±0.59	0.35±0.100	0.075±0.034	0.027±0.0060
prednisone	0.01	3.52±0.35	0.28±0.047*	0.05±0.011**	0.02±0.0043*

The data shown in the tables 1.1, 1.2, 1.3, 1.5 and 1.6 prove that the Fengshiping can strongly inhibit the primary and secondary injury caused by FCA, whenever the medicine was given at the beginning of the FCA injection or 2 weeks after the FCA injection. The experiments prove that the Fengshiping has both the preventive and the therapeutic effect. By comparing the effect of Fengshiping on the degree of swelling of the anklejoint and foot, we found that the Fengshiping can inhibit the specific immuno-swelling of the ankle joint better than the nonspecific immuno-swelling in the foot of rats. This result indicates that the main effect of Fengshiping was inhibiting the inflammatory reaction of the immune system.

The data in the tables 1.3, 1.4 and 1.7 show that the AA rats had no obvious BW (body weight) increase during the period of the experiment. In the group given the Fengshiping with the effective dosage, the rats continued to increase in BW. In the groups treated with prednisone, the BW of rats decreased, while the thymus and adrenal gland atrophied. In the group treated with *tripterygium hypoglaucum* (Levl.) Hutch, the

thymus had not atrophied. In the 3 groups given the different dosage of Fengshiping, no atrophy of the thymus and adrenal gland were observed.

1.3 The pathologic change of the AA after the treatment of the invented medicine in rats

Forty-five (45) SD rats, 180±20g weight each, were divided into 6 groups. After the AA caused by FCA appeared, all the rats were given orally, Fengshiping solution for 5 days once a day. One (1) hour after the last administration, the joint index of the rats was measured and calculated. The damaged joints of the posterior limbs distal from the FCA injection were removed and soaked in formaldehyde. After the tissues were stained with HE (hematoxylin-eosin), the pathological change of the synovium and cartilage were observed and recorded. The data are shown in table 1.8.

1.8 The effect of Fengshiping on the AA joint index in the rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Rat number	Joint index
Control	-	8	0**
AA model	-	7	6.2±0.49
Fengshiping	7.5	9	4.86±0.90**
Fengshiping	15	7	4.71±0.95**
Fengshiping	30	7	4.56±1.13**
Glucosidorum Tripterygll Totorum	0.006	7	4.57±0.79**

Comparing with the model groupP<0.01**

The joint index was the sum of the inflammatory scores of the four limbs. According to the degree of inflammatory, each limb was evaluated on the criteria as following: normal (0), red without swelling (1), red and swelling (2), seriously swelling (3), deformity and stiffness

(4).

Observed from the microscope, the joint synovial membranes of the rat posterior limb were hyperplastic in the control group; the collagen fiber had increased; and there was infiltration of lymphocytes and plasma cells in the tissue. An obvious granuloma had formed. The synovial cells had degenerated and the cytochylema was tinted red; the nucleus had become pycnotic; the epithelium had exfoliated in some part of the synovial membrane. The cartilage became atrophied; its surface was rough and some of the chondrocytes had proliferated slightly.

After the treatment with the Fengshiping, the inflammation of the joint synovial membrane was inhibited, more collagen fiber was produced; less synovial cells exfoliated ; the cells on the surface of the cartilage had proliferated and the surface had turned smooth. The cartilage was in a recovering condition.

Experimental example 2: The effect of Fengshiping on the delayed typed hypersensitivity reaction (DTH) caused by 2,4-DNFB (dinitrofluorobenzene) administered to the ear of the mouse

Fifty (50) NIH mice, half male and half female, were divided into 5 groups. Each mouse was induced into hypersensitivity by application of 1% DNFB acetone solution at a dosage of 0.025ml at the right side of the abdomen where fur had been removed. Using the same solution at the same place enhanced the reaction on the third day. On the fifth day, all the mice were smeared with the 1% DNFB edible oil solution at the mice's right ears at a dosage of 0.01 ml each. Twenty-four (24) hours later, all the mice were killed. The mouse's 2 ears were weighed by the torsion balance and the difference of the 2 ears was recorded as the

degree of DTH caused by the DNFB. The experiment was carried out using different immune-inhibitory compounds and administration processes.

2.1 The effect on the DTH by the full course administration

The immune and administration processes is as follows:

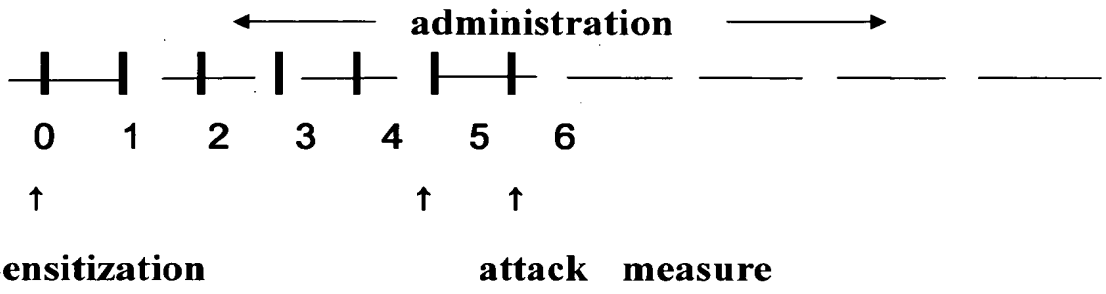

Table 2.1 The effect of Fengshiping on the DTH caused by DNFB in the NIH mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Administration time (day)	Mice number	Percent of ear swelling	Percent of inhibition (%)	P value
control			10	34.20±3.77		
Fengshiping	27	0~5	10	26.24±3.34	23.3	<0.01
Fengshiping	40	0~5	10	12.99±4.96	62.0	<0.01
Fengshiping	60	0~5	10	10.43±7.53	69.5	<0.01
cortisumman	0.003	0~5	10	13.93±4.41	59.3	<0.01
control			10	42.43±5.28		
Fengshiping	40	-2~0	10	31.50±10.52	25.0	<0.01
Fengshiping	40	-2~2	10	30.88±7.92	27.2	<0.01
Fengshiping	40	-2~5	10	21.07±4.62*	50.3	<0.01
Fengshiping	40	5~6	10	32.00±9.37	41.7	<0.01
cyclophosphane	0.05	-2~2	10	39.40±10.78	8.1	>0.05
cyclophosphane	0.05	-2~0	10	37.47±6.71	11.7	>0.05
control			10	38.50±4.67		
cyclophosphane	0.1×3	0、2、4 day once a day	10	23.00±7.65	40.3	<0.01
cyclophosphane	0.25	-3d	10	41.84±7.75	-8.7	
Fengshiping	60	0~4	10	27.20±10.20	29.4	<0.01
cyclophosphane +Fengshiping	0.25 + 60	-3,0~4	10	38.07±6.65	1.1	

*comparing with the other groups P<0.05或P<0.01

The data shown in table 2.1 indicate that the Fengshiping had an obvious inhibitory effect on the DTH caused by DNFB. There was a significant relationship between the dosage and the effect. The inhibitory activity increased when the dosage increased. The inhibitory effect could reach 69.5% at a dosage of 60.9g/kg.

2.2 The effect on the DTH with the different administration time

The corresponding results from using different immune-inhibitory compounds and administration processes are shown in the middle and bottom parts of table 2.1. According to the results shown in the middle part of the table 2.1, all the different administration can significantly inhibit DTH of the mouse regardless whether the administration began from 2 days before the sensitization to the end of sensitization, or whether it began from 2 days before the sensitization to 2 days after the end of sensitization, or whether it began 2 days before the sensitization to 5 days after the end of sensitization, or whether it began before the attack and end after the attack. However, the administration that began 2 days before the sensitization and ended 5 days after the sensitization had the most powerful inhibitory activity. The data indicate that the Fengshiping could inhibit the DTH by multiple mechanisms: it can inhibit the cells ~~t~~participating in the early period of the DTH, and it can inhibit the effector cells in the latter period as well as cells related to the DTH in the middle period. This mechanism of inhibition by Fengshiping was different from that of the cyclophosphane. Using a small dosage, the cyclophosphane did not affect the DTH, if its administration began 2 days before the sensitization and ended at the day of sensitization or 2 days after the day of sensitization.

Based on the bottom part of the table 2.1, if a high dosage of cyclophosphane was given to the mouse at one time 3 days before the sensitization, the function of the Th (T helper) cells would become sthenic because of the powerful inhibition on the Ts (suppressor T) cells. The DTH in the mouse would be enhanced. If the cyclophosphane was used with the Fengshiping in this administration method, it could lower the inhibitory activity of Fengshiping. This result indicates that the Fengshiping has a different machnism compared to the cyclophosphane in the control of DTH. Fengshiping may have a higher activity in inhibiting Th cells.

Experimental example 3: The effect on the humoral immunity

3.1 The effect on the levels of the hemolysin antibody initiated by the CRBC (chicken red blood cells)

One hundred ninety (190) mice, 18-22g weight, half male and half female, were divided into 19 groups. Each mouse was immunized with 0.2 ml of 5% CRBC solution. Fengshiping solutions were given orally to the mice at the different times. Seven (7) days after the immunization, blood samples from all the mice were taken from the eyes. Then the blood samples were diluted and the levels of the hemolysin antibody were measured. The results are shown in table 3.1, 3.2 and 3.3.

Table 3.1 The effect of Fengshiping on the levels of the hemolysin antibody in the NIH mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Administration time	Mouse number	Hemolysin value	Inhibitory percent (%)	P value

control				10	169.0±62.0		
Fengshiping		18	0~7	10	46.0±15.6	72.8	<0.01
Fengshiping		27	0~7	10	35.4±12.0	79.1	<0.01
Fengshiping		40	0~7	10	28.2±5.9	83.3	<0.01
Fengshiping		60	0~7	10	16.7±3.0	90.1	<0.01
Tripterygium hypoglaucum Hutch.	(Levl.)	13.3	0~7	10	121.0±88.0 **	28.4	<0.015
cyclophosphane		0.02	0~7	10	35.0±12.0	79.3	<0.01

**** comparing with the Fengshiping (40g/kg) group P<0.01**

Table 3.2 The effect of Fengshiping on the levels of the hemolysin antibody in the ICR mouse ($\overline{X} \pm S$)

group		dose (g/kg)	Administration time	Mouse number	Hemolysin value	Inhibitory percent (%)	P value
control		-	-	10	124.70±42.60		
Fengshiping		12	0~7	10	75.00±53.10	39.9	<0.05
Fengshiping		18	0~7	10	45.60±22.70	63.4	<0.01
Fengshiping		27	0~7	10	29.10±22.10	76.8	<0.01
Fengshiping		40	0~7	10	28.20±5.30	77.4	<0.01
Tripterygium hypoglaucum Hutch.	(Levl.)	6.0	0~7	10	143.50±67.90**		>0.05
cyclophosphane		0.02	0~7	10	27.80±6.60	77.9	<0.01

****comparing with the Fengshiping (18g/kg) group P<0.01**

Table 3.3 The effect of Fengshiping on the levels of the hemolysin antibody in the ICR mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Administration time	Mouse number	Hemolysin value	Inhibitory percent (%)	P value
control	-	-	10	256.0±26.0		
Fengshiping	18	-7~7	10	198.0±50.0	22.7	<0.01
Fengshiping	18	-3~7	10	156.0±85.0	39.1	<0.01
Fengshiping	18	0~7	10	98.0±35.0	61.7	<0.01
cyclophosphane	0.02	0~7	10	25.0±4.0	90.2	<0.01

According to the data in table 3, Fengshiping has a remarkable inhibitory effect on the levels of the hemolysin antibody in the different mouse species and this effect would increase along with an increase of

the dosage. There was a certain relationship between the dosage and the effect. The lowest effective dosage was 12g/kg. Compared with the same quantity of Tripterygium hypoglaucum (Levl.) Hutch, the Fengshiping had a higher inhibitory activity. Based on the data in table 3.1, the inhibitory activity of Fengshiping was 2.25 times higher than the Tripterygium hypoglaucum (Levl.) Hutch. The inhibitory activity of Tripterygium hypoglaucum (Levl.) Hutch. with the dosage of 13.5g/kg was weaker than that of the Fengshiping which contains 6g/kg Tripterygium hypoglaucum (Levl.) Hutch).

3.2 The effect of the Fengshiping on the humoral immunity in the AA mouse

The NIH mice, 20±2g weight, were injected with 0.05 ml of FCA under the skin of the right posterior limb. Three (3) weeks later the AA model mouse was established. The model mice were divided into 6 groups randomly and given orally the corresponding medicines for 5 days. At the beginning of the administration, all the mice were sensitized with 0.5ml of 10% sheep RBC (SRBC). Five days later, all the mice were killed. Their spleens were taken out and washed with Hank's buffer to prepare the lymphocyte in suspended solution. The concentration of the cells was adjusted to 2×10^7 / ml. One (1) ml of lymphocyte suspension, 1 ml of 0.2% SRBC and 1 ml of 1:30 addiment were added to one test tube. The tube was put in the water bath at 37℃ for 1 hour. Then the tube was centrifugated at 2000rpm for 5 minutes. The supernatant fluid was separated and tested for its optical density at the 415nm wavelength on a spectrophotometer. The value was representative of PFC (plaque-forming cell) quantity.

The other portion of the blood samples from the sensitized mice was used to isolate the serum to test the potency of the antibody. The measured data were recorded using Log2 value. (See the data in table 3.4)

Table 3.4 The effect of Fengshiping on the humoral immunity in the mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Mouse number	PFC (OD)	IgM(Log2)
control	-	8	0.819±0.013#	6.875±0.641
AA model group	-	10	0.940±0.019**	7.700±0.599*
fengshiping	5	8	0.834±0.012**#	6.875±0.641#
fengshiping	10	8	0.834±0.012**#	6.750±0.886#
fengshiping	20	8	0.830±0.014**#	6.375±0.518##
Glucosidorum Tripterygll Totorum	0.012	10	0.835±0.015**#	6.950±0.597#

Comparing with the control group *P<0.05, **P<0.01; comparing with the model group # P<0.05, ## P<0.01

According to the table 3.4, the levels of PFC and IgM in the AA mouse were higher than that of the normal mouse. Fengshiping can lower the levels of the PFC and IgM in the AA mouse significantly.

Experimental example 4: The effect of Fengshiping on the passive cutaneous anaphylactic reaction (PCA) in the rat.

The rats were injected with the egg albumn at 10mg/kg in the muscle. At the same time, all the rats were injected with 2×10^{10}(0.2ml) bordetella pertussis in the abdominal cavity for sensitization. Two (2) weeks later, all the rats were killed to sample the blood. All the blood samples were separated to obtain the serum.

Sixty (60) rats, 150~200g, half male and half female, were divided into 6 groups at random. In the light narcosis condition induced by ether,

each rat was shaved on its back and injected with the 2x 0.1 ml concentrations of anti-egg-album serum under the shaved area of the skin. The serums were diluted to concentrations of 1:5(d1) and 1:10(d2) before the experiment. Forty-eight (48) hours later, all the rats were intravenously injected with 0.5% Evans blue normal saline solution 1 ml which contained 1 mg egg albumin. Twenty (20) minutes later, the rats were killed by decapitation. The skins on the rats' back were dissected and examined. According to the dark and light areas of the blue stains exudated from the vessels, all the rats were evaluated by several people. The skins stained by the Evans blue were scissored and soaked in 5ml of 0.1% sodium sulfate acetone (7:3) solution for 48 hours. Then it was centrifuged to separate the supernatant liquid. The optical density of the supernates was measured at the wavelength 590nm to calculate the degree of the PCA reaction and the inhibitory percent. The results were shown in table 4.

Table 4 The effect of Fengshiping on the PCA in rat ($\overline{X} \pm S$)

Group	dose (g/kg)	value		absorbancy	
		d_1	d_2	d_1	d_2
Control	-	5.60±1.78	2.40±2.46	0.191±0.129	0.096±0.106
Fengshiping	12	7.50±2.51	4.20±2.49	0.402±0.213*	0.192±0.175
Fengshiping	24	7.10±2.13	4.10±1.79	0.310±0.177	0.137±0.099
Fengshiping	48	6.00±1.83	1.70±1.95	0.121±0.109	0.024±0.026*
Tripterygium hypoglaucum (Levl.) Hutch.	8	6.11±1.27	2.56±1.67	0.223±0.122	0.074±0.045
Ketotifen	0.1	2.78±1.64**	0.67±1.41	0.033±0.024**	0.027±0.019*

Comparing with the control group *P<0.05 , **P<0.01

The data in table 4 indicate that the Fengshiping had a weak effect on the PCA in the rat. Only at a high dosage was the inhibitory effect of Fengshiping obviously different from that of the control group.

Experimental example 5: The effect of Fengshiping on the cytokines.

5.1 The effect of Fengshiping on the levels of TNFα and IL-2 in the mouse.

Sixty (60) ICR mice, 18~22g, half male and half female, were divided into 6 groups at random. Each group was given orally the corresponding medicines including the different dosages of Fengshiping and the other medicines. The medicines were administrated once a day for 10 days. Twenty-four (24) hours after the last administration, samples from the mice were taken, including the macrophages and spleen cells from the abdominal cavity in the aseptic condition. The samples were washed with Hank's buffer twice and with non-serum RPIM 1640 liquor once. Then the washed samples were diluted to a suspension with 5% FCS-RPMI 1640 at a concentration of 2×10^8 cells / ml. Then the suspensions were added with 10ng/ml LPS (lipopolysaccharide) or the 10ng/ml ConA (concanavlin A) and cultured in the 5% CO2 condition for 48 hours at 37℃. Then the TNF α and IL-2 levels in the suspensions were measured using the usual methods.

The measurement of TNFα

The plate was coated with mouse TNF-α monoclonal antibody. The plate had cultured supernate added at the dose of 50μl/well. Then the plate was incubated for 60 minutes at room temperature. Then the plate was mixed with biotin-tagged antibody marker at 25℃ for 2 hours. Then the avidin-tagged enzyme (e.g., horseradish peroxidase, one that cleave or react against a detection substrate) was added into the plate and incubated for 30 minutes. After adding the substrate for the enzyme for

30 minutes, the stop solution was added to the plate. The mixed liquid was measured using the OD value at the wavelength of 450nm. The content of the TNF-α(ng/ml) was calculated based on the OD value compared with standard curve.

The measurement of the IL-2:

The CTLL (cytotoxic T lymphoid cells) cells which were on the logarithmic growth phase and whose growth depends on IL-2, were adjusted in a suspension to a concentration of 1×10^5 cells/ml with 5% FCS-RPMI 1640. Then the 96-well cell culturing plate was filled with the CTLL cell suspension at a volume of 100μl/well. Each sample was added in triplicate. To measure the concentration of IL-2, the cultured suspensions were compared with different dilutions of standard rHIL-2 (recombinant human interleukin 2) in the control sample (culture fluid). All the samples were incubated in 5% CO2 for 24 hours at 37℃. Six (6) hours before the end of the incubation, all the samples were centrifuged and separated from the supernate. Each well had 110 μl of supernate removed and then 10 μl of MTT was added to each well. The samples were cultured for 3 hours at 37℃, and then the OD was measured at the wavelengths of 570nm and 630nm. The final OD value of the sample was the difference of OD (570nm) and OD (630nm).

$$\text{IL-2 activity} = \frac{\text{Sample}\overline{\text{OD}} - \text{Control (Culture Fluid)}\overline{\text{OD}}}{\text{Standard Sample}\,\overline{\text{OD}} - \text{Control (Culture Fluid)}\overline{\text{OD}}} \times \text{activity of the standard sample (IU/ml)}$$

Table 5.1 The effect of Fengshiping on the TNF αnd IL-2 ($\overline{X} \pm S$)

group	dose (g/kg)	Mouse number	TNF (pg/ml)	IL-2 (IU/ml)
Control	-	10	87.80±14.63	26.30±4.22
Fengshiping	12	10	62.14±13.13**	16.00±2.89**
	24	10	58.60±9.63**	18.80±2.86**
	36	10	54.40±10.88**	18.20±2.86**
Tripterygium hypoglaucum (Levl.) Hutch.	8	10	58.25±10.32**	16.00±2.88**
cyclophosphane	0.02	10	42.20±9.57**	10.10±3.00**

*P<0.05 , **P<0.01

The data in Table 5.1 s uggest that Fengshiping has an obvious inhibitory effect on the levels of TNFα. At a dosage of 12g/kg, the medicine had shown an obvious inhibitory effect. With an increase in dosage, the inhibitory effect duly increased. But the dosage-effect curve was gradual. Fengshiping had an obvious inhibitory effect on the levels of IL-2, but no dosage-effect relationship was observed.

5.2 The effect of Fengshiping on the IL-1, IL-6

Seventy (70) NIH mice, 18-22g weight, half male and half female, were divided into 7 groups at random. All the groups were given orally the corresponding medicines (Fengshiping and other medicines). The medicines were given once a day for 10 days. Twenty-four (24) hours after the last administration, all the mice were killed and macrophages and spleen cells from the abdominal carvity were sampled. The IL-1 and IL-6 in the samples were measured.

The measurement of IL-1:

The macrophages in the abdominal cavity were sampled in a sterile condition. Then the samples were washed with Hank's buffer twice and nonserum RPMI1640 media once. Then the washed samples were

adjusted to 4×10^6 cells / ml cell suspension with 5% FCS-RPMI media. One (1) ml of the suspension was added to the test tube and cultured at 37°C for 1 hour. The nonadherent cells were discarded. Then 5% FCS-RPMl 1640 and LPS (10ng/ml) were added to cell culture. The cells were cultured in 5% CO_2 at 37°C for 72 hours. Afterwards, the cultured cells were freezed and thawed several times. The final product was saved at 4°C. Thymuses from C57 mice were obtained in sterile condition. Then the samples were adjusted to 1×10^6 cells/ml cell suspension with 5% FCS-RPMl1640.

One hundred (100) μl supernate separated from the freeze-thawed solution and 100μl cell suspension of the thymus were added to 96-well flat bottom cell-culture plates. Each sample was cultured in triplicate and compared with the different dilutions of the standard rHIL-1 and the control sample (culture fluid). Each well had 2ng ConA added and then the plate was cultured in 5% CO_2 at 37°C for 72 hours. Fourteen (14) hours before the end of the culture, each well was added 3H-TdR (tritiated thymidine) at 0.1μCi. The cultured cells were collected with a multihead cell-harvesting apparatus and the cpm (count per minute) value was measured.

$$\text{IL-1activity} = \frac{\overline{\text{Sample cpm}} - \overline{\text{Control (Culture Fluid) cpm}}}{\overline{\text{Standard Sample cpm}} - \overline{\text{Control (Culture Fluid) cpm}}} \times \text{activity of the standard (ng/ml)}$$

The measurement of the IL-6:

The spleen cells were sampled in sterile condition. Then the samples were washed with Hank's buffer twice and nonserum

RPMI1640 media 1 time. Then the clear samples were adjusted to 2×10^6 cell/ml cell suspension with 5% FCS-RPMI media. One (1) ml of the suspension was added to a round-bottom centrifuge tube. After adding the ConA (10ng/ml), the samples were cultured in 5% CO_2 at 37℃ for 72 hours.

The MH60 cells, their growth dependent on IL-6 and at the logarithmic growth stage, were adjusted to 1×10^5 cells/ml cell suspension with the 5% FCS-RPMI1640.

The 96-well flat bottom cell culturing plate was added the MH60 cell suspension at 100μl/well and the culturing suspension of spleen cells at 25μl/well. Then the volume in each well was adjusted to 200μl with 5% FCS-RPMI 1640. Each sample was cultured in triplicate and compared with solutions at different concentrations of standard rHIL-6 and the pure culturing fluid. The plate was incubated in 5%CO_2 at 37℃ for 72 hours. Six (6) hours before the end of the incubation, the samples were centrifuged. In each well, 110μl of the supernate was sucked out and 10μl of MTT was added. The samples were kept at 37℃ for 3 hours. And then the OD at the wavelength 570nm and 630nm were measured. The final OD value = OD 570nm – OD 630nm.

$$\text{IL-6 activity} = \frac{\text{SampleOD - Culturing Fluid ControlOD}}{\text{Standard SampleOD - Culturing Fluid ControlOD}} \times \text{Sample Dilution} \times \text{Activity Of The Standard (IU/ml)}$$

Table 5.2 The effect of Fengshiping on the IL-1, IL-6 ($\overline{X} \pm S$)

Group	Dose (g/kg)	Mouse number	IL-1 (ng/ml)	IL-6 (IU/ml)
Control	-	10	78.7±7.1	94.6±6.8
Fengshiping	7.5	10	59.3±4.9**	64.9±4.8**
	15	10	53.3±5.7**	60.5±4.3**
	30	10	54.4±4.8**	56.0±4.6**
	60	10	47.0±16.6**	56.6±6.1**
Tripterygium hypoglaucum (Levl.) Hutch.	5	10	57.6±4.7**	65.7±4.9**
cyclophosphane	0.02	9	44.5±7.7	49.6±6.7**

Based on the data in the table 5.2, Fengshiping had an obvious inhibitory effect on the macrophage in producing IL-1 and spleen cell in producing IL-6. With increase in dosage, the effect is duly enhanced.

5.3 The effect of Fenghsiping on the plasma NO (nitric oxide) in the AA rat

Sixty (60) SD rats, 160 ~ 220g weight, half male and half female, were divided into 6 groups. The rats in the blank control group were injected with 0.5ml of NS (normal saline) under the skin of the right posterior hindlimb. Other rats were injected with 0.5ml of FCA at the same place as that of the control group. Eighteen (18) days later, the AA model was established. Then the rats were given orally the corresponding medicines or distilled water once a day for 5 days. Three (3) groups were given orally the solution of Fengshiping at high, middle and low dilution. The positive control group was given orally Glucosidorum Tripterygll Totorum. The blank control group and the model group was given orally distilled water of the same volume. One (1) hour after the last administration, 2 ml of each rat's blood from the abdominal aorta was sampled. The plasma of the blood samples was separated and saved at - 70℃ for measurement. The measurement of

NO was done as per the directions of standard NO-detection kit. Based on the OD value of the sample, the content of NO was calculated on the standard curve. (See the result in table 5.3)

Table 5.3 The effect of Fengshiping on the plasma NO level in the AA rat ($\overline{X} \pm S$)

Group	Dose (g/kg)	Rat number	Content of NO (μmol/L)	y (y=Lgx)
Control	-	8	13.55±1.11*	1.131±0.032
AA model	-	9	17.56±4.15	1.235±0.097
Fengshiping	12	7	9.83±2.58**△△	0.985±0.087
Fengshiping	24	7	10.12±1.56**△△	1.001±0.067
Fengshiping	48	7	10.70±1.51**△△	1.026±0.062
Glucosidorum Tripterygll Totorum	0.006	7	15.25±3.48	1.173±0.099

Comparing to the model group*P<0.05 , **P<0.01 ; comparing to the Glucosidorum Tripterygll Totorum△△P<0.01

Based on the data in table 5.3, the NO level was higher in the model group than in the blank control group. Fengshiping had an obvious effect on lowering the NO level in the AA rat. The Glucosidorum Tripterygll Totorum had similar effect but its effect was weaker than that of Fengshiping.

Experimental example 6 : The effect of Fengshiping on T lymphocyte, CD_4, CD_8 and NK cells in the mouse.

6.1 The effect of Fengshiping on the transformation of lymphocytes in the normal mouse.

Eighty (80) NIH mice, half male and half female, were divided into 8 groups randomly and given orally the corresponding medicines once a day for 10 days. Twenty-four (24) hours after the last administration, all

the mice were killed to sample the spleen cells aseptically. Then the samples were washed with Hank's buffer twice and nonserum RPMI1640 media once. Then the washed samples were adjusted to 2×10^6 cells/ml cell suspension with 5% FCS-RPMI media. The 96-well flat bottom cell culturing plate was added the cell suspension at a volume of 100μl/well. Each sample was cultured in triplicate. Of the triplicate, two (2) wells were added 2ng of ConA each as the stimulating reagent. The third well was not given ConA and was kept as the control well. The plate was incubated in 5% CO_2 at 37℃ for 72 hours. Fourteen (14) hours before the end of the incubation, each well had 3H-TdR 0.1μCi added. The cells were harvested using the multihead cell harvesting instrument and were measured for their cpm. The average value was adopted as the sample's cpm. The average cpm and the stimulating index of the different groups were compared directly. The stimulating index was calculated as follows:

$$\text{Stimulating Index} = \frac{\text{Stimulated } \overline{\text{cpm}}}{\text{Control } \overline{\text{cpm}}}$$

See the result in table 6.1

Table 6.1 The effect of Fengshiping on the lymphocyte transformation induced by ConA in the mouse ($\overline{X} \pm S$)

Group	Dose (g/kg)	Mouse number	cpm	Stimulating index
Control	-	10	20433±3579	25.87±3.06
Fengshiping	7.5	10	13566±1779**	27.29±7.67
	15	10	12708±1692**	18.04±3.76
	30	10	12809±2575**	16.17±4.37
	60	10	12090±1706**	19.05±3.80
Tripterygium hypoglaucum (Levl.) Hutch.	2.5	10	18038±3359	17.11±2.60
	5	10	12081±1039**	17.58±4.37
Cyclophosphane	0.02	9	9922±1145**	13.66±2.28

Comparing to the control group*P<0.05 , **P<0.01

The data in table 6.1 indicate that Fengshiping had an obvious inhibitory effect on the lymphocyte transformation and there was a dosage-effect relationship.

6.2 The effect of Fengshiping on the CD_4, CD_8 and NK cells

The experiment was same as the experiment described in 5.1. Twenty-four (24) hours after the last administration, the spleen cell samples were made into a 2×10^8 cells/ml cell suspension with 5% FCS-RPIMl640. The quantity of CD_4, CD_8, NK cells and the rate CD_4/CD_8 were measured by the usual method.

The measurement of CD_4 and CD_8:

Fifty (50) μl of the spleen cell suspension was added to a glass to smear the cell. The glass had been coated with polylysine. The T cell of the mouse was set as the positive control sample. The cell smear was enveloped by the serum of the normal mouse after it was fixed with acetone. Then the enveloped sample was incubated with antibodies to CD_4 and CD_8 tagged with hominine biotin. It was incubated at 37°C for 2 hours. Then avidin-tagged enzyme enzyme (one that can cleave or react against a detection substrate) was added to the sample and incubated for 10 min. After the substrate was added for 10 min, the mixed sample was washed and dyed with hematoxylin for 2 min. Then the sample was dehydrated with the grade-alcohol and enveloped with gelatin-glycerol. Two hundred (200) cells in the smear were chosen to be evaluated under the high power microscope.

$$\text{Content Of Cell} = \frac{\text{Dyed cell number}}{200} \times 100\%$$

The measurement of the NK cell:

The preparation of the EC cell: The spleen cells were sampled in sterile condition. Then the samples were washed with Hank's buffer twice and nonserum RPMI1640 media once. Then the clear samples were adjusted to 2×10^8 cells/ml cell suspension with 5% FCS-RPMI media. This cell suspension was used as the EC.

The preparation of the TC cell: The Yack-1 cells, which were sensitive to the mouse NK cell and growing at the logarithmic phase, were adjusted to 4×10^4 cells/ml cell suspension. This cell suspension was used as the TC.

Measurement: EC and TC, One hundred (100) μl each were added to the 96-well flat bottom cell culturing plate. Each sample was cultured in triplicate with 2 control samples: EC and TC. (EC control: EC100μl + 5% FCS RPMI 1640 100μl ; TC control : TC100μl + 5% FCS RPMl 1640 100μl). The samples were incubated in 5% CO_2 at 37℃ for 24 hours. Six (6) hours before the end of the incubation, the samples were centrifuged and 110μl supernate were aspirated out of each well. And then 10μl of MTT were added to each well. After incubating at 37℃ for 3 hours , the OD values of the mixed samples were measured at the wavelengths of 570nm and 630 nm. The OD of each well =OD570nm - OD630nm.

$$\text{Activity Of NK} = \left(1 - \frac{\text{Sample } \overline{OD} - \text{EC Control } \overline{OD}}{\text{TC Control } \overline{OD}}\right) \times 100\,\%$$

Table 6.2 The effect of Fengshiping on the CD4, CD8, NK cell ($\overline{X} \pm S$)

Group	Dose (g/kg)	Mouse number	CD4 (%)	CD8 (%)	CD4/CD8	NK
Control	-	10	20.80±2.94	14.80±2.49	1.42±0.18	40.13±4.89
Fengshiping	12	10	19.14±2.91	13.43±2.51	1.43±0.08	31.94±4.52**△△
	24	10	17.30±2.51**	12.00±2.40	1.46±0.16	35.36±3.40*△△
	36	10	16.30±2.50**	11.23±2.94**	1.49±0.20	31.06±3.53**△△
Tripterygium hypoglaucum (Levl.) Hutch.	8	10	16.25±2.25**	11.50±2.45	1.44±0.18	32.20±2.00**
Cyclophosphane	0.02	10	11.50±2.50**	4.10±1.20**	2.91±0.53**	23.10±3.66**

Comparing to the control group*P<0.05 , **P<0.01 ; comparing to the cyclophosphane△△P<0.01

According to the table 6.2, Fengshiping had some inhibitory effect on CD_4 cells and CD_8 cells. There was a relation between the dosage and the effect, but the dosage-effect curve was gradual. The effective dosage of Fengshiping on the inhibition of CD_4 was 24g/kg. The minimum effective dosage on inhibiting the CD_8 was 36g/kg. Fengshiping had no obvious effect on the ratio of CD_4/CD_8. Cyclophosphane had an obvious effect in inhibiting both kind of cells with the inhibitory effect on the CD_8 was very potent, which could increase the ratio of CD_4/CD_8 significantly.

As for NK cell, Fengshiping had a remarkable inhibitory effect, but the dosage-effect relationship was not certain. Similarly, cyclophosphane had shown an obvious inhibitory effect on the NK cell. At a dosage of 20mg/kg, the inhibitory effect of cyclophosphane was significantly different from that of Fengshiping at its 3 dosages: 12, 24 and 36g / kg.

6.3 The effect on the transformation and function of the T lymphacyte in the AA mouse.

NIH mice, 20±2g weight, were injected with 0.05 ml of FCA under the skin of the right posterior limb to build the AA model. The mice in the control group were injected with 0.05ml NS at the same place. Three (3) weeks later, after the AA model was built, all the mice were given orally the corresponding medicines once a day for 5 days. Five (5) days later, all the mice were sampled, and the blood was used to make blood smears. The smears were dyed by esterase. Then the smears were observed under an oil immersion lens to calculate the percent of the positive-dyed cells (it represented the content of the T cells in the blood). The spleen cells of the mice were sampled while the mice were under

anaesthesia and then the cell samples were prepared in a cell suspension. The cell suspension was washed by PBS and then its supernate was discarded. The rest had 4 ml of blood cell lysate added. The mixed sample was shaken for 2～3 min to solubilize the RBC. After the RBCs were solubilized, the sample was centrifuged to separate and discard the supernate. The sample without supernate was washed with the luminescence solution twice. Then it was centrifuged to separate and discard the supernate. In the next step, the sample was adjusted to the 1×10^6 cells/ml cell suspension. Each tube was added 50μl of diluted antibody to CD_4 and CD_8. . Then the tubes were incubated at 4℃ for 1 hour. After the culture, the samples were washed with the luminescence solution twice and 2ml of the fixing fluid was added. After fixing, the samples were filtrated through the 400-mesh screen to the FCA tube. The filtrated samples were analyzed by flow cytometer (FCM). The result is shown in table 6.3.

Table 6.3 The effect of Fengshiping on the T cell in the AA mouse

$(\overline{X}\pm S)$

Group	Dose (g/kg)	ANAE+ (%)	CD4+ (%)	CD8+ (%)	CD4+/CD8+
Control	-	50.60±4.25	26.13±1.16	15.56±0.68	1.68±0.03
AAmodel	-	49.00±4.22▲	32.56±2.87**	13.59±1.03**	2.49±0.16**
Fengshiping	7.5	49.13±4.03▲	27.30±1.76##▲	15.98±1.11##▲	1.71±0.04##▲
	15	49.31±3.29▲	27.96±1.67##▲	16.23±1.27##▲	1.73±0.05##▲
	30	48.56±3.23▲	26.75±1.94##▲	15.58±1.29##▲	1.72±0.04##▲
Glucosidorum Tripterygll Totorum	0.012	48.88±2.89▲	27.88±1.99##▲	16.33±1.31##▲	1.70±0.03##▲

n=8 , comparing with the control group*P<0.05 , **P<0.01 ; comparing with the model group# P<0.05 , ## P<0.01 ; comparing with

the control group▲P>0.05

According to the data in table 6.3, there was no significant difference in the different groups on the ANAE (alpha- naphthyl acetate esterase) positive cell. But in the AA mouse, the increase of the CD_4 was significant, while the decrease of CD_8 was significant too. So the ratio of CD_4/CD_8 had a remarkable increase. The result indicates that Fengshiping could adjust the CD_4, CD_8 and CD_4/CD_8 to the normal range.

Experimental example 7: The effect of Fengshiping on the phagocytic function of the macrophage in the mouse abdominal carvity.

Fifty (50) NIH mice, 18~ 22g weight, half male and half female, were divided into 5 groups and given orally the corresponding medicine solutions at the same volume. The administration was once a day for 7 days. One (1) hour after the last administration, all the mice were injected with 0.2ml 10 % chick RBC into the abdominal cavity. Four (4) hours later, all the mice were killed and the fluid in the abdominal cavity was sampled. The liquid samples were deposited on the glass and the number of macrophages was counted that had phagocytized the CRBC and the number of the CRBC in each macrophage was also counted. (See the result in table 7)

Table 7 The effect of Fengshiping on the CRBC phagocytosis function of the macrophage in ICR mouse abdominal cavity ($\overline{X} \pm S$)

group	dose (g/kg)	Mouse number	Percent of phagocytosis (%)	phagocytosis index
Control	-	10	25.75±9.40	1.28±0.20
Fengshiping	27	10	33.20±12.77	1.46±0.36
Fengshiping	40.5	10	35.20±10.16	1.21±0.20
Fengshiping	60.9	10	37.78±20.14	1.53±0.32
dexamethasone	0.005	10	8.33±10.13*	1.10±0.18

*P<0.05

According to the table 7, Fengshiping had no obvious effect on the phagocytotic function of the macrophage in the mouse abdominal cavity.

Experimental example 8: The effect of Fengshiping on the hyperfunction of the capillary permeability in the mouse abdominal cavity.

Ninety (90) NIH mice, 18~22g weight, half male and half female, were divided into 9 groups and given orally the corresponding medicine solutions of the same volume. The medicines were given once a day for 3 days or just 1 time. One (1) hour after the last administration, each mouse was injected with 0.7% HAC (acetic acid) – NS solution into the abdominal cavity. At the same time, each mouse was injected with the 0.5% Evans blue – NS solution into the vessel at a dose of 0.1ml/10 g. Thirty (30) min later; all the mice were killed by cervical dislocation. The abdominal cavity was opened and washed with 5ml NS. The NS used was collected and adjusted to a volume of 8ml by pure NS to be used as the sample. The samples were centrifuged at 3000 rpm to get the supernate. The supernate OD was measured at the wavelength of 590nm. (See the result in table 8)

Table 8 The effect of Fengshiping on the hyperfunction of the capillary permeability induced by the acetic acid in the mouse abdominal cavity ($\overline{X} \pm S$)

Group	Dose (g/kg)	Administration	Mouse number	Leakage of the tincture (OD)	P value
Control	-	-	10	0.29±0.13	
Fengshiping	27	qd×1	10	0.26±0.14	>0.05
Fengshiping	40	qd×1	10	0.25±0.10	>0.05
Fengshiping	60	qd×1	10	0.25±0.09	>0.05
Control	-	-	10	0.28±0.15	
Fengshiping	27	qd×3	10	0.25±0.12	>0.05
Fengshiping	40	qd×3	10	0.18±0.10	<0.05
Fengshiping	60	qd×3	10	0.15±0.13	<0.05
dexamethasone	0.15	qd×3	10	0.11±0.07	<0.01

The data in table 8 indicate that Fengshiping could obviously inhibit the hyperfunction of the capillary permeability induced by the acetic acid in the mouse abdominal cavity if the mouse was given the medicine for 3 days continuously. If the medicine was given for just once, the inhibiting effect was not obvious.

Experimental example 9: The effect of Fengshiping on the pleuritis exudation and aggregation of the inflammatory cell induced by the carrageenan.

The mice were divided into 5 groups at random and injected with 0.5% Evans blue NS solution into the caudal vein at a dosage of 0.1ml/10g. Then the mice were injected with the 0.03ml 1% carrageenan in the right chest cavity with a syringe needle. Four (4) hours and 32 hours after the injection, the corresponding mice were killed and had their abdominal cavity opened to expose the diaphragm. Two (2) ml of the solution were injected to the chest cavity twice with a 1 ml injector. The solution was collected and saved in a test tube. Twenty (20) μl of the

solution collected was added into the 400μl WBC dilution. The WBC in the mixed dilution was counted under the microscope. The rest of the solution was centrifuged at 3000rpm for 10 min. The supernate was measured for its OD at a wavelength of 600nm. The OD value of the sample should be corrected with the correspondent OD value of the pure solution. (See the result in table 9)

Table 9 The effect of Fengshiping on the inflammatory cell aggregation induced by the carrageenan ($\overline{X} \pm S$)

Group	Dose (g/kg)	WBC number(2×105)		Tincture exudation (OD)	
		4h	32h	4h	32h
Control	-	46.0±6.9	16.0±9.6	0.156±0.066	0.109±0.019
Fengshiping	27	26.8±4.5*	14.2±8.0	0.121±0.062	0.116±0.031
Fengshiping	40.5	10.9±4.0**	17.3±4.6	0.100±0.048	0.153±0.032
Fengshiping	60	8.0±5.5**	6.6±4.7*	0.129±0.066	0.092±0.051
dexamethasone	0.05	12.7±10.2**	4.4±4.0*	0.085±0.045	0.063±0.017

*P<0.05 , **P<0.01

The data in table 9 indicate that Fengshiping had an obvious inhibitory effect on the inflammatory cell aggregation. The effect was powerful at the early stage. The regression equation on the data of the fourth hour was as follows: y=44.13 - 2.01x , r= - 0.9625. The effect at the late stage was weak. At the high dosage of 20g/kg, the medicine could affect the aggregation of the WBC. But it had no obvious effect on the pleuritis exudation.

Experimental example 10: Effect on aggregation of leukocyte in rats with a sac of CMC (carboxymethylcellulose).

Sixty four (64) SD rats, 150-180g weight, half male and half female, were randomly divided into 8 groups, and they were given orally the same volume but different dosage of drug liquid once a day for 3

days. A day before experiment, rats were injected with 20ml 1% CMC solution into the sac at the rat's back created by 20ml air injection before the experiment. Three and a half hours and 7.5 hours later, 0.1ml of liquid in the sac was extracted each time, and was colored with 0.01% brilliant cresyl blue solution and leukocytes were counted in the sac liquid under a microscope. The results are shown in the table 10.

Table 10 effect on leucocyte counts of carboxymethyl cellulose sac of rats with Fengshiping ($\overline{X} \pm S$)

groups	dosage (g/kg)	rats number	WBC count(×107/L)	
			3.5 hrs	7.5 hrs
control	-	8	9.7±4.2	57.7±17.3
Fengshiping	27×1	8	8.5±3.5	39.4±16.5
Fengshiping	40×1	8	8.7±7.3	35.3±23.2
Fengshiping	60×1	8	6.6±3.3	18.1±8.6**
Control	-	8	10.97±6.7	35.6±11.2
Fengshiping	27×3	8	15.4±9.7	38.6±15.5
Fengshiping	40×3	8	4.8±3.4**	18.4±12.2**
Fengshiping	60×3	8	3.0±2.8**	11.0±9.2*
cortisone	0.1×3	8	14.2±8.0	41.7±16.0
Control	-	8	10.9±3.0	41.3±6.9
Fengshiping	18×7	8	6.2±3.0*	11.4±6.4*
Fengshiping	27×7	8	3.7±1.7**	6.4±3.1**
Fengshiping	40×7	8	2.5±1.9**	5.9±3.9**
cortisone	2mg×1	8	1.5±0.7**	3.0±1.0**

Compared with control group**P<0.01

According to table 10, Fengshiping could inhibit significantly aggregation of leucocyte in the rats' sac containing CMC, and the inhibition showed apparent dosage-effect correlation, which was stronger with greater frequency of administration. With administration continuing seven days, the migration of leucocyte could be inhibited significantly at dosage of 18g/kg. Similarly, there was also very strong inhibition with cortisone injection into the sac.

Experimental example 11: The effect on croton oil-induced swelling in the ears of mice.

Sixty (60) NIH mice with weight of 18~22g, male and female accounting for half and half, were divided into 6 groups, which were given orally with the same volume and different dosage of drug liquid or tragacanth liquid, once a day, lasting 3 days. One (1) hour after the final administration, 2% croton oil mixure of 0.02ml was embrocated uniformly on both sides of the left ears of the mice, and after 4 hours, the mice were put to death by snapping their cervical vertebra. The left and right ears were cut down, then the inflammed and control ears were weighed by certain means. Differences in weight between left and right ears reflect the extent of the swelling of the inflammed ears, with results shown in table 11.

Table 11 Effect on croton oil-induced swelling of the ears of mice with Fengshiping ($\overline{X} \pm S$)

Groups	dosage (g/kg)	rats number	Degree of ears' swelling (mg)	inhibition rate (%)	P value
Control group	-	10	44.38±9.40		
Fengshiping	27	10	39.05±12.33	12.00	>0.05
Fengshiping	40	10	36.65±5.83	17.64	<0.05
Fengshiping	60	10	34.91±9.71	21.34	<0.05
dexamethasone	0.003	10	14.13±5.75	68.16	<0.01

As seen from table 11, Fengshiping had remarkable inhibition to croton oil-induced swelling of the ears of mice, and had dosage-effect correlation, but the curve was gradual. There was significant inhibition effect at 13.5g/kg of dosage.

Experimental example 12: Effect on acetic acid-induced twisting reaction of mice.

Sixty (60) Kuming mice with weight of 18~22g, male and female

accounting for half and half, were randomly divided into 6 groups, which were given orally different dosages of drug liquid or tragacanth solution. One (1) hour after administration, 0.2ml of 0.7% HAC saline solution was injected, sc (subcutaneous), and the mice were placed in an enclosure and observed for the length of the latent period before the twisting reaction of each mouse and the twisting times in 20 minutes, with the results shown in table 12:

Table 12 The effect of Fengshiping on acetic acid-induced twisting reaction of mice ($\overline{X} \pm S$)

groups	dosage (g/kg)	Rats numbers	Twisting times	Latent time (minute)
Control	-	10	34.6±14.1	3.13±0.80
Fengshiping	27	10	28.2±5.76	3.82±0.85
Fengshiping	40	10	31.0±18.4	3.86±2.00
Fengshiping	60	10	20.7±12.3*	3.95±1.42
Tripterygium hypoglaucum (Levl.) Hutch.	20	10	25.1±11.9	3.60±0.93
morphine hydrochloride	10mg/kg	10	0.0±0.0	0.00±0.00

The data from table 12 indicate that large doses of Fengshiping could delay the latent time before the HAC-induced twisting reaction and significantly reduce the twisting times in 20 minutes, which indicated Fengshiping had the effect of aberration in some degree.

Experimental example 13: Effect on hemorheology of AA rats.

Each SD (Sprague Dawley) rats, 180±20g weight, were injected intracutaneously with 0.05ml Freund's complete adjuvant on the right back foot metatarsal, and they were developed into adjuvant arthritis models. Each of the rats of negtive control group were injected intracutaneously with 0.05ml saline on the right back foot metatarsal. Three weeks after models were built, the rats were divided into model group, large, middle, small dosage groups, negtive control group and

positive control group which was administered with Glucosidorum Tripterygll Totorum. The rats were given the medicines orally once a day, lasting 5 days. One (1) hour after administration for the last time, 3ml of blood was taken from abdominal aorta of rats and placed into test tube with 1% heparin as an anticoagulant, and the whole blood viscosity was measured at shear rates of 230, 115, 46, 23, 11.5, $5.75S^{-1}$ with an NXE-1 cone and plate viscometer. The plasma viscosity was measured with a WTP-BII adjustable constant pressure capillary viscosimeter. The haematocrit, erythrocyte aggregation index was measured with the centrifugation method of packed cell volume. The rigidity index was calculated from the above-mentioned data. All the results are shown in table 13.

Table 13 Effect on hemorheology of adjuvant arthritis model rats ($\overline{X} \pm S$)

Groups	Control group	Model group	Fengshiping (30g/kg)	Fengshiping (15g/kg)	Fengshiping (7.5g/kg)	Glucosidorum Tripterygll Totorum (6mg/kg)
whole blood viscosity (mPa.s)						
230S-1	4.43±0.09	4.92±0.15**	4.56±0.09##	4.49±0.11##	4.54±0.16##	4.66±0.28#
115S-1	5.17±0.25	5.81±0.19**	5.33±0.09##	5.32±0.10##	5.16±0.14##	5.60±0.48#
46S-1	6.84±0.11	7.20±0.18**	6.56±0.13##	6.59±0.09##	6.67±0.14##	6.70±0.48#
23S-1	8.10±0.15	8.23±0.38	7.95±0.22	7.93±0.12	7.97±0.14	8.02±0.14
11.5S-1	9.35±0.08	9.78±0.10**	9.40±0.08##	9.45±0.10##	9.30±0.133	9.31±0.12##
6.5S-1	11.03±0.14	12.66±0.31**	11.21±0.21##	11.29±0.19##	11.60±0.40##	11.42±0.52##
Plasma viscosity (mPa.s)	1.158±0.032	1.248±0.040**	1.161±0.011##	1.154±0.023##	1.156±0.018##	1.158±0.029##
corpuscular volume (%)	46.13±2.31	41.33±1.12**	45.10±2.39##	44.33±1.52##	45.71±1.04##	46.03±3.59##
erythrocyte aggregation index	2.49±0.032	2.58±0.083*	2.46±0.066#	2.49±0.094#	2.44±0.048##	2.45±0.091#
rigidity index	6.155±0.536	7.127±0.557**	6.506±0.558	6.525±0.146	6.394±0.200#	6.621±0.883

Compared with negative control group*P<0.05 , **P<0.01 ; compared with model control group# P<0.05 , ## P<0.01

According to the table 13, the hemorheology of AA rats was changed significantly compared with control rats. The whole blood and plasma viscosity increased, haematocrit decreased, aggregation index and rigidity index of erythrocyte increased. Fengshiping could significantly improve the above-mentioned indexes of hemorheology.

Pharmacological effects of Fengshiping have been proven by the above-mentioned experiments. Many important pharmacological effects of Fengshiping had favorable dosage-effect correlation, which implied the best therapeutic effectiveness might be obtained by adjusting the drug dosage at the clinical level.

The clinical studies on Fengshiping were carried on in China, Japan and Austrilia. Theses studies were done according to international criterion related disease classification regarding diagnosis, therapy and curative effect. The effective rate for RA was around 94%, and its notable effective rate was around 60%. It could improve the symptoms such as morning stiffness, swelling and pain and other related items. The results showed in tables 14 ~ 21.

Table 14 Compared effect of treatment group with control group

Groups	Cases	remission (clinical recovery)	Notable effect	Effective	No effect	Notable effect rate (%)	Effective rate (%)
Treatment group	32	5	14	11	2	59.38	93.74
Control group	30	3	10	12	5	43.33	83.33

Table 15 Influence of IgG, IgA and IgM ($\overline{X} \pm S$)

Groups	cases	IgG		IgA		IgM	
		pre -	post -	pre -	post -	pre -	post -
Normal	32	12.45±1.48		2.37±1.00		1.58±0.59	
Treatment group	32	16.92±3.49	14.17±1.39**	3.65±1.03	2.39±1.18**	1.89±0.88	1.48±1.01
Control	30	17.03±4.12	15.14±2.21**	3.45±1.86	2.32±1.75**	2.03±0.95	1.76±1.28

Comparing with pre-treatment **P<0.01

Table 16 Influence of C3 and C4($\overline{X} \pm S$)

groups	cases	C3		C4	
		pre -	post -	pre -	post -
normal group	32	0.62±0.13		0.14±0.15	
Treatment group	32	1.88±0.72	1.25±0.66**	0.48±0.12	0.26±0.06*
Control group	30	2.13±0.64	1.56±0.62**	0.40±0.16	0.25±0.07**

Comparing with before therapy *P<0.05, **P<0.01

Table 17 Influence of ESR and CRP ($\overline{X} \pm S$)

Groups	cases	ESR		CRP	
		pre-	post-	pre-	post-
Normal	32	8.37±5.26		4.12±1.88	
Treatment	32	66.58±9.01	30.31±6.53**	13.35±6.67	8.86±3.34*
control	30	73.33±9.09	35.83±11.61**	14.21±6.29	9.04±3.15**

Comparing with pre-treatment *P<0.05, **P<0.01

Table 18 Compared with power of gripping pre- and post-treatment ($\overline{X} \pm S$)

groups	Treatment Group		Control Group	
	pre -	post -	pre -	post -
Gripping power of left hands (mmHg)	39.13±20.24(15)	80.47±34.61**(15)	24.00±17.63(21)	55.15±23.27**(21)
Right hands	35.85±22.46(15)	85.32±36.32**(15)	22.80±12.32(21)	58.17±20.59**(21)

Comparing with pre-treatment *P<0.05, **P<0.01

Table 19 Influence of arthrosis swelling and pain and morning stiffness time ($\overline{X} \pm S$)

Items	Treatment Group		Control Group	
	pre -	post -	pre -	post -
arthrosis swelling and pain	5.79±0.52	3.14±0.83*	5.56±2.15	3.92±0.26*
morning stiffness time (minute)	50.33±6.47	20.24±3.27**	48.75±8.34	27.50±3.78**

Comparing with pre-treatment *P<0.05, **P<0.01

Table 20 Influence of RF changing to negative

Groups	Cases	RF negative		
		Pre - treatment	Post - treatment	Rate of negative turnaround (%)
Treatment group	32	24	11	54.2
Control group	30	18	10	44.4

Not only did it show significant effects for the above items in tables 14-20, but Fengshiping can also decrease the levels of items such as SIL-2R, STNF, SIL-6R in plasma as shown in the Table 21.

Table 21 inflence of main indes such as SIL - 2R, STNF αnd SIL - 6R ($\overline{X} \pm S$)

groups	Cases	SIL - 2R(u/ml)		STNF R1(ng/ml)		SIL - 6R(ng/ml)	
		pre -	post -	pre -	post -	pre -	post -
Normal	32	299±68 (n=32)		1.56±0.48 (n=24)		72.05±18.26 (n=22)	
Fengshiping	15	683±189	381±157**	2.87±0.66	1.75±0.54**	136.18±28.57	90.15±20.12**
Control	10	765±203	412±167**	2.63±0.72	2.38±0.39 (n=8)	148.21±30.31	99.02±26.70**

Comparing with pre-treatment **P<0.01

It was proven in the above-mentioned results that the invention can be realized by the ways as follows:

Example of use 1:

Epimedium brevicornum Maxim. 2222g

Tripterygium hypoglaucum (Levl.) Hutch. 2222g

Lycium barbarum L. 1111g

Cuscuta chinensis Lam. 1111g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted for three times with 13, 10, 10-volume water , each time lasting 1 hour; *Epimedium brevicornum* Maxim was cut into segments, extracted three times with 15, 10, 10-volume water, each time lasting 1 hour; *Lycium barbarum* L. was crushed into coarse powder, and immersed in 20-volume water of 80℃ for 1 hour; *Cuscuta chinensis* Lam. was crushed into coarse powder, immersed in 31-volume water of 80℃ for 1 hour; the decoction fluid or immersion fluid of four herbs were filtrated respectively, poured into column with polymeric adsorbent resins having macropores, and eluted with 70% ethanol. When the color of eluent became deep significantly, eluent was collected until the color of eluent became very weak, at which time the elution collection ended. The alcohol in the eluent of each herb was reduced. Then the fluid without alcohol was concentrated and dried to get the final extract powder; officinal starch was blended with the four kinds of extract powder to 200g, mixed uniformly and encapsuled into 1000 capsules. Each capsule which was prepared with the invented method thereof, was composed of 0.2g drug extract and contained at least 2.0mg of icariin $C_{33}H_{40}O_{15}$. The regular dosage is: oral administration, three times every day, three capsules each time.

Example of use 2:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-volume water, each time lasting 1 hour; *Epimedium brevicornum* Maxim. was cut into segments, extracted three times with 15, 10, 10-volume water, each time lasting 1 hour; decoction fluid of herbs were filtrated respectively, poured into column with polymeric adsorbent resins with macropores, eluted with 70% ethanol, when the color of eluent became deep significantly, eluent was collected until eluent became very weak, at which time the elution ended. The alcohol in the eluent of each herb was removed. Then the fluid without alcohol was concentrated, dried to get the final extract powder; officinal starch was blended with the extracted drug powder, and mixed uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive, contains at least 2.0mg of icariin $C_{33}H_{40}O_{15}$. regular dosage is: oral administration, three times every day, three capsules for each time.

Example of use 3:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Lycium barbarum L. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-times water, each time lasting 1 hour; *Epimedium brevicornum* Maxim.was cut into segments, extracted three times with 15, 10, 10-times water, each time lasting 1 hour; *Lycium barbarum* L. was crushed to coarse powder, and immersed in 20-times water of 80°C for 1 hour; decoction fluid or immersion fluid of four herbs were filtrated respectively, poured across a macropore polymeric

adsorbent column, eluted with 70% alcohol, when the color of effluent became deep significantly, eluent was started to be collected; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with the extractive drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive, contains at least 2.0mg of icariine $C_{33}H_{40}O_{15}$. Regular dosage is: oral administration, three times every day, three capsules each time.

Example of use 4:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Cuscuta chinensis Lam. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-volume water , each time lasting 1 hour; *Epimedium brevicornum* Maxim.was cut into segments, extracted three times with 15, 10, 10-volume water, each time lasting 1 hour; *Cuscuta chinensis* Lam. was crushed to coarse powder, immersed in 31-volume water of 80°C for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated respectively, poured across a macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of eluent began; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was reduced. Then the fluid without alcohol was concentrated, and dried to obtain the final extract powder; officinal starch was blended with extract drug powder, and mixed uniformly,

loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drug extract, contains at least 2.0mg of icariin $C_{33}H_{40}O_{15}$. Regular dosage is: oral administration, three times every day, three capsules each time.

Example of use 5:

Tripterygium hypoglaucum (Levl.) Hutch. 2000g

Cuscuta chinensis Lam. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-volume water, each time lasting 1 hour; *Cuscuta chinensis* Lam. was crushed to coarse powder, immersed in 31-volume water of 80℃ for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated respectively, poured across the macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of the eluent began; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried, to obtain the final finally extract powder; officinal starch was blended with extractive drug powder, and mixed up uniformly, and loaded to 1000 capsules. The dose of capsules administered every day, which was prepared with the inventive method thereof, was equivalent to 30g of crude drugs.

Example of use 6:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Lycium barbarum L. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-volume water , each time lasting 1

hour; *Lycium barbarum* L. was crushed to coarse powder, and immersed in 20-volume water of 80℃ for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated respectively, poured across macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of the eluent began; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to obtain the final extract powder; officinal starch was blended with extractive drug powder, and mixed up uniformly, and loaded to 1000 capsules. The dose of capsules administered every day, which was prepared with the inventive method thereof, was equivalent to 30g of crude drugs.

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